RyR1/Caveolin-3 interaction, to analyze the functional consequences of the disruption of this interaction, and the possible origin of the pathology.

3205-Pos Board B310
Sarcoplasmic Reticulum Ca^{2+} Release in Mouse Muscle Fibers Expressing Pathological Mutant Forms of the Type 1 Ryanodine Receptor
Romain Lefebvre, Claude Legrand, Estela Gonzalez-Rodriguez, Linda Groom, Robert T. Dirksen, Vincent Jacquemond.
Mutations of the gene encoding type 1 ryanodine receptor (RyR1) are associated with susceptibility to malignant hyperthermia (SMH) and/or central core disease (CCD). We used in vivo expression of EGFP-RyR1 DNA constructs to study the function of pathological forms of RyR1 in fully differentiated muscle fibers. The Y522S, R615C, R2163H and I4897T mutant RyR1s were separately expressed in mouse *flexor digitorum brevis* muscles, using plasmid injection and electroporation. All EGFP-RyR1s were present within restricted regions of the transfected fibers with a pattern consistent with triadic localization. Confocal measurements of voltage-activated rhod-2 Ca^{2+} transients demonstrated spatially confined alterations of SR Ca^{2+} release closely correlated with the presence of the mutant channels; in fiber regions expressing Y522S-RyR1 channels, Ca^{2+} release was specifically enhanced at low and moderate levels of depolarization: for instance peak Ca^{2+} release in response to pulses from −80 to −30 mV and −10 mV was 3.0 ± 0.6 and 1.5 ± 0.3 (n=9) times larger in the Y522S-positive fiber regions than in adjacent negative ones, respectively. When expressing wild-type EGFP-RyR1s, the corresponding ratio value was 0.86 ± 0.1 (n=9) at the two same voltages. Results similar to the ones with Y522S were observed with R615C and R2163H RyR1s. Conversely, expression of I4897T-RyR1s tended to be associated with a reduced SR Ca^{2+} release: for instance peak value at −10 mV was depressed by 45 ± 10 % (n=6). Results are thus consistent with an inherent increased sensitivity to activation by the voltage sensor of SMH-associated RyR1 mutant channels and an inherent functional failure of the CCD-associated I4897T ones. Overall, the present strategy proves to be highly effective to reveal the dysfunction of SR Ca^{2+} release due to mutant-RyR1s in differentiated muscle fibers.

3206-Pos Board B311
Modulation of Sarcoplasmic Reticulum Ca^{2+} Release by Phosphatidylinositol-Phosphate Lipids in Isolated Mouse Skeletal Muscle Fibers
Estela Gonzalez-Rodriguez, Romain Lefebvre, Karine Poulard, Claude Legrand, Anna Buj-Bello, **Vincent Jacquemond**.
Previous results from ryanodine binding and single ryanodine receptor (RyR) channel measurements suggested that RyR1 activity may be modulated by phosphatidylinositol-phosphate lipids (PtdInsPs). Possibly related, recent data showed that skeletal muscle e-c coupling is altered in several models of PtdInsPs phosphatase deficiency. We measured intracellular Ca^{2+} in fibers from *flexor digitorum brevis* (fdb)muscles microinjected with a solution containing a given PtdInsP lipid (PtdIns(3,5)P2, PtdIns(3)P, PtdIns(5)P or PtdIns) together with the calcium dye indo-1. Following equilibration, fibers were stimulated by short voltage-clamp depolarizations of increasing amplitude from −80mV. Resting [Ca^{2+}] did not differ between control fibers and any group of PtdInsPs-injected fibers; it was though ~1.5 larger in fibers injected with PtdIns(3,5)P2 or PtdIns(3)P than in fibers injected with PtdIns(5)P or PtdIns. Peak SR Ca^{2+} release was specifically depressed in fibers injected with PtdIns(3,5)P2 or PtdIns(3)P with maximum values of 34.8 ± 3 $\mu M.ms^{-1}$ in control fibers (n=15) and of 24.4 ± 2 and 17.4 ± 2 $\mu M.ms^{-1}$ in fibers injected with PtdIns(3,5)P2 (n=8) and PtdIns(3)P (n=8), respectively. There was no concurrent effect on the membrane current measured during the pulses and the voltage dependence of Ca^{2+} release inactivation was also unaffected. These results highlight the possibility that SR Ca^{2+} release may be physiologically regulated by certain PtdInsPs. A plasmid coding for a wild-type form of the PtdIns phosphatase MTM1 tagged with the red fluorescent protein mCherry was transfected in fdb muscles. The protein was found distributed within the entire volume of the transfected fibers and to reproducibly yield a striated transversal expression pattern consistent with localization within the triadic area. Preliminary measurements of voltage-activated Ca^{2+} transients in these cells indicated that under these conditions over-expression of MTM1 had limited effects on SR Ca^{2+} release.

3207-Pos Board B312
Culture Methods and Initial Characterization of Calcium Homeostasis in Intercostal Skeletal Fibers Isolated from the Adult Mouse
Patrick Robison, Erick O. Hernández-Ochoa, Martin F. Schneider.
Primary cultures of single adult skeletal muscle fibers dissociated from locomotor muscles adhered to glass coverslips are routine models which allow monitoring of functional processes in living cultured fibers under controlled conditions. To date, such isolated fiber cultures have not been established for respiratory muscles, despite the critical function of these muscles and their role in mortality of many muscular diseases. We have developed an adherent culture system for single adult intercostal muscle fibers from the adult mouse. This system allows for monitoring functional properties of these living muscle fibers in culture exposed to various conditions (pharmacological agents, electrical field stimulation to drive muscle fiber contraction, etc.). We also provide initial characterization and demonstrate several common electrophysiological, Ca2+ imaging techniques and over-expression of foreign fluorescent fusion proteins in this new model system with comparisons to the established Flexor Digitorum Brevis muscle primary culture model. Supported by NIH-NIAMS Grants R01-AR055099, R01-AR056477 and T32-AR007592.

3208-Pos Board B313
Muscle Performance and Electrically Evoked Ca^{2+} Release are Compromised in Calsequestrin-1 Null Mice
Rotimi Olojo, Erick O. Hernández-Ochoa, Paul D. Allen, Martin F. Schneider, Christopher W. Ward.
In skeletal muscle the sarcoplasmic reticulum (SR) Ca2+ release channels (i.e., ryanodine receptor; RyR1) are critical determinants of contractile filament activation. Recent evidence suggests that several SR proteins may modulate RyR dependent SR Ca2+ release and thus could alter overall function. Recent attention has been focused on the SR luminal protein calsequestrin (Casq) as a SR Ca2+ buffer as well as its potential role in modulating the RyR1, the DHPR and other sarcolemmal channels. In our current work, we tested the hypothesis that mice null for Casq1 will have functional impairments reflecting the role of Casq1 in fast type skeletal muscle. Here we examined functional measures of overall muscle performance in vivo and of fast muscle in vitro, and identified significant deficits in functional performance that indicate an inability to sustain repetitive contractile activation. We then used measures of voltage dependent SR Ca2+ release and SR Ca2+ release flux in single fast type skeletal muscle fibers. Here we demonstrate a decrease in voltage dependent RyR Ca2+ release with single action potentials and a collapse of the Ca2+ release with repetitive trains of pulses. Finally in single voltage clamped muscle fibers we show that SR Ca2+ release flux as well as total SR Ca2+ release is markedly reduced in the Casq1 null myofibers. The voltage dependence of the Ca2+ release flux was not shifted but showed about 50% decrease in the maximum peak Ca2+ release flux in the Casq1 null fibers when compared to the WT counterpart. Taken together we have revealed that the genetic depletion of Casq1 results in significant deficits in contractile activation consistent with alterations in SR Ca2+ release. Supported by RC2 NR011968, R01-AR055099, and T32- AR007592.

3209-Pos Board B314
Measurement of Intra-SR [Ca^{2+}] Reveals Changes in SR Ca^{2+} Permeability During Intracellular Ca^{2+} Release in Skeletal Muscle
Monika Sztretye, Jianxun Yi, Leandro Royer, Jingsong Zhou, Lourdes Figueroa, Paul D. Allen, Eduardo Rios.
In skeletal muscle, Ca2+ release from the SR is under strong regulation. While this regulation has well known Ca2+-dependent components acting from the cytosolic side of the channels (CICR and CDI), putative effects of luminal calcium are not well defined. Combining imaging of $[Ca^{2+}]_{SR}$ by the cameleon D4cpV targeted with calsequestrin 1 or its Ca^{2+}-impaired variant "Delta-Asp", with measurements of cytosolic [Ca^{2+}] (X-rhod 1) in single mouse FDB fibers, we directly monitored the evolution of Ca^{2+} permeability (P) of the SR membrane during long-lasting voltage clamp depolarizations. These depolarizations were intended to both elicit the release response and substantially deplete the SR of calcium, so that P could be evaluated as the SR calcium decreased. Against our expectations (Royer 2007; 2010) P was found to decrease significantly during depolarization. Consequently, depletion was never complete, and $[Ca^{2+}]_{SR}$ reached on average 40% of the resting content after long pulses. This gating effect of depletion is similar to that which in cardiomyocytes is believed to terminate Ca^{2+} release and be associated with the presence of calsequestrin. Similar measurements in cells from casq1-null mice showed instead > 90% depletion during long-lasting pulses. Permeability remained essentially constant, at a high value, for the duration of the pulses. This was confirmed under various compositions of cytosolic and external solutions, with different calsequestrin-derived targeting sequences and widely varying biosensor expression densities. An inhibitory effect on Ca^{2+} release is evidently lost in the casq1-null mouse. Whether this reflects allosteric gating by calsequestrin remains to be established. The observation goes a long way towards explaining the modest effect of calsequestrin KO in the maximal amount of SR calcium that can be released by depolarization (Royer, 2010).
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